Report Title: Delayed Contact Hypersensitivity in Guinea –Pigs Modified by Ritz, H. L. and Buehler, E. V. on E-2414.01(ECM BTS 930) according to P&G Protocol No. C 4, June 1983.

Test Type: Delayed Contact Hypersensitivity

Conducting Laboratory and Location: International Bio-Research Europe

Test Substance(s): #E2414.01 – 1% Octopirox in shampoo. Undiluted material used for testing.

Species: Guinea pig

# of Animals:

Test Conditions: Modified Buehler method. 0.3 ml applied in Hill Top Chambers at 25, 50, 75 and 100% in preliminary study to find highest non-irritating concentration. In the main study, 50% tested.

Results: Preliminary study showed slight to moderate erythema at 100% and 75%. No evidence of delayed contact hypersensitivity in main studied tested at 50%.

Study #: IBRE-1-3-599-84; IBRE-1-3-600-84
Report Date: 10/19/84

Accession #: 31973

BCM BTS : 930

BIOLOGICAL TEST FOR SAFETY : EUROPEAN OPERATIONS REQUEST DOCUMENT (EORD).

A

Originator	A. Cairns	17/5/84	Originator's SH	T.C. Bell	24/5/84	Originator's AD	R.E. Atkinson
	Name	Date		Name	Date		Name Date
Toxicologist	D.J.G. Müller	3/5/84	PARS SH	G. Calvin	3.5.84	PARS Manager	T.M. McCarthy 28/5/84
	Name	Date		Name	Date		Name Date

Estimated Total Cost : £ 1400

Estimated Dates of : Availability of Analytical Data : _____

Receipt of Report : Summer 1984

Product Coordination Manager : J.B. Camden 29/5/84 (Name Date)

B

Name of Test Substance : Head & Shoulder AR w/ 1% Octopirox

Identification Number E-2414.01

Substances required by (date) _____

Agreed by PDP _____

TSCR Completion Date _____

	Panel/Blind Test	Ship Test	Test Market	National
Type of Consumer Exposure(s)for Which Safety Clearance is Requested				
No. of Subjects	200			
Starting Date	fall '84			
Duration	6 weeks			
Location	UK			

C

Safety Test Requirements (By PARS)

Tests :

	Amount of Substance Needed	Cost
1. LD_{50} (rat), Up & Down method	200 g	£ 300
2. Eye Irritation (rabbit, low volume protocol)	20 g	£ 250
3. Skin Irritation (rabbit)	20 g	£ 100
4. Skin Sensitization (guinea Pig)	100 g	£ 750

D.J.G. Müller
Toxicologist

3-5-84
Date

/mp.w - 0229M

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November 1984 / et

Project-No.: 2-5-598-84

RECEIVED : 10-12-1984

CHECKED BY: 19-12-84

Delayed Contact Hypersensitivity in Guinea-pigs modified by Fitz, H. L. and Buehler, E. V. on M-2414.01 (SCN 81TS 930) according to P & G Protocol-No. C 4, June 1983

Head & Shoulders AR w/ Octopirox

Sponsor:
Procter & Gamble
European Technical Center
Temselaan 100
B - 1820 Strombeek-Bever (Strombeek-Bever)

CONTENTS

I. INTRODUCTION

It was the objective of this study to determine whether "E-2414.01" (ECM ETS 930) causes delayed contact hypersensitivity in guinea-pigs under occluded conditions.
For the performance of this study the Procter & Gamble protocol no. C 4 (issue date June 16, 1983) was followed.

II. SUMMARY

a) The investigation on delayed contact hypersensitivity was done in 2 groups of male and female guinea-pigs.

All signs of erythema and edema were recorded after a 3-week induction period and a primary challenge two weeks later.

b) The preliminary study showed slight to moderate erythema (100 % and 75 %).

c) On all animals no signs of erythema and edema were observed.

According to the method modified by Ritz, H. L., and Buehler, E. V., the test substance "E-2414.01" (BCM BTS 930) is considered to cause no delayed contact hypersensitivity.

We, the undersigned, hereby declare that the work was performed under our supervision and according to the procedure herein described. The described symptoms, findings and data correspond to the results obtained.

Dr. Dr. W. Sterner .....

Fachtierarzt für
Pharmakologie u. Toxikologie

Expert agréé
pharmacologue, toxicologue

Fachtierarzt für
Klin. Laboratoriumsdiagnostik

Fachtierarzt für
Versuchstierkunde

Dr. G. Chibanguza .....

Fachtierarzt für
Pharmakologie u. Toxikologie

Stilkampen, November 30, 1984

III. MATERIAL AND METHOD

1. Animals

1.1	Species:	Guinea-pigs
1.2	Strain:	Pirbright
1.3	Substrain:	Hoe: DHPK (SPF-LAC.) /Boe.
1.4	Source:	Lippische Versuchstierzucht Hagemann GmbH & Co. KG Hamelner Straße 3 4923 Extertal 1
1.5	Colour:	white
1.6	Background of strain:	Originally bred at Duncan Hartley's, England. Erection of a SPF-strain after hysterectomie at the LAC (Laboratory Animal Center, GS). Hoechst, Frankfurt, continued breeding animals that were descended from LAC after hysterectomie. 1976 the breeder received animals from Hoechst, with which he continued breeding under SPF-conditions.
1.7	Date of receipt:	04.10.84
1.8	Acclimatization time:	7 days at least
1.9	Randomization:	by the way of lottery drawing
1.10	Animal identification:	earmark and/or colour identification Cage card with the following information: damage, sex, (ear-mark), test, day of the beginning of the study.
1.11	Weight range at the beginning of the study:	≙ 330 g

2. Husbandry

2.1	Caging:	two animals in one cage
2.2	Cagetype:	Macrolon Plastic cages III 14 cm high, 25 cm width, 42 cm length
2.3	Lighting:	Fluorescent light, 4000° K, 120 Lux
2.4	Lighting periods:	12 hours daily, from 7.00 a.m. to 7.00 p.m.
2.5	Temperature:	18° C ± 2° C
2.6	Relative humidity:	50 - 85 %
2.7	Registration:	by thermohygrometer
2.8	Timing:	in the morning and in the afternoon

3. Food and feeding

3.1 Producer: Semiff Spezialfutter GmbH
4770 Soest/Westfalen

3.2 Name: Semiff-G (Alleindiät für Meerschweinchen)

3.3 Type: pellets, 1.0 cm large, 0.5 cm diameter

3.4 Composition:

crude nutritive substance:	crude protein	21,0 %
	crude fat	3,5 %
	crude fibre	15,0 %
	crude ash	8,2 %
	humidity	12,0 %
	N-free extract agent	39,0 %
metabolisable energy:	Kcal/kg	2680
	kJ/kg	11218
Amino acid:	Lysin	1,20 %
	Methionin	0,30 %
Vitamins:	24.000 I.U.	Vitamin A
	2.000 I.U.	Vitamin D_3
	2.000 mg	Vitamin C
	45 mg	Vitamin E
Minerals and Trace elements:	Ca	1400 mg
	P	900 mg
	Na	200 mg
	Mg	200 mg
	K	13200 mg
	Cl	8400 mg
	Fe	230 mg
	Mn	55 mg
	Cu	16 mg
	Zn	36 mg
	S	4600 mg
	Co	300 mcg
	J	350 mcg

4. Bedding

4.1	Producer:	Sniff Spezialfutter GmbH 4770 Soest/Westfalen
4.2	Name:	Sniff - Bedding
4.3	Production:	from pure spruce-, fir- and pine-wood, dried and disdusted
4.4	Sterilization:	180° C
4.5	Water binding capacity (% of bedding used):	276,5

5. Water

5.1	Administration:	ad libitum
5.2	System:	Macrolon drinking bottles, 300 ml, Fa. Becker & Co., 4620 Castrop-Rauxel
5.3	Quality:	Aqua fontana as for human consumption
5.4	Quality control:	regular analysis by an official laboratory for water analysis.

6. Test material

The test substance "E-2414.01" (BCM BIS 930) was supplied by Procter & Gamble European Technical Center, Grimbergen (Strombeek-Bever), Belgium.

6.1	DRD-Number:	BCM BIS 930
6.2	General characteristics:	"E-2414.01" (BCM BIS 930) is a blue, turbid, strong viscous liquid.
6.3	Storage:	room temperature

7. Experimental design

7.1 Preparation of the animals

Following an acclimatization period of 7 days at least to accustom the guinea-pigs to the environmental conditions existing in our laboratories, the test was initiated.
The animals were allocated in two groups (1 testgroup and 1 controlgroup), the testgroup contains 20 animals and the controlgroup 10 animals. Equal numbers of male and female guinea-pigs were used. They were marked by colour identification.
Prior to treatment the left shoulder of each animal was clipped with a small animal clipper.

7.2 Preliminary study

In the course of a preliminary test, the highest non-irritating concentration was determined.
Therefore the entire back and both sides of 8 animals were clipped one day prior to application.
The following day the animals were exposed for one 6-hour period to various concentrations of the test substance. The sample was applied in 4 different concentrations: 100 %, 75 %, 50 % and 25 % in aqua dest.. The responses were graded at 24 hours and at 48 hours according to the procedure described below (refer to 7.6).

7.3 Preparation of the test substance

a) Preliminary study

The sample was applied 100 % (= undiluted), 75 %, 50 % and 25 % in aqua dest..

b) Main study

Referring to the preliminary study, the test sample "E-2414.01" (ECH ETS 930) was applied 50 %.

7.4 Treatment

Induction of Sensitization

The day before exposure the left shoulder of each animal was clipped with a small animal clipper.
0.3 ml of the freshly prepared test substance was applied to the "Hill Top Chambers". These were placed on the clipped surface of each animal and secured with several wrappings of plastic material. The animals were immobilized in restrainers for 6 hours. After that time the patches were taken off and the test substance was removed with a gentle rinse of warm water (about 37° C) before returning the animals to their cages.
The procedure was repeated at the same site once a week for the next two weeks for a total of three 6-hour exposures. After the last induction exposure the animals were left untreated for 2 weeks before primary challenge.

Primary challenge

The animals previously exposed during the induction period as well as the previously untreated animals were treated following the same patching procedure with the "Hill Top Chambers" as for the induction, but the patches were applied to a freshly clipped skin site (right shoulder), that has not been treated before.

7.5 Observations

24 hours after the primary challenge all animals were depilated with Pilca Cream (used for cosmetical depilation, produced by Olivin, Hamburg). The depilatory was used according to the instructions of the producer. The test sides were graded 6, 24 and 48 hours after the depilation.

7.6 Scores

No reaction	0
Slightly patchy erythema	±
Slight, but confluent or moderate patchy erythema	1
Moderate erythema	2
Severe erythema with or without edema	3

Grades of 1 or greater in the test group indicate sensitization, provided grades of less than 1 are seen on the control animals. If grades of 1 or greater are noted on control animals, then the reactions of the test animals that exceed the most severe control reaction are presumed to be due to sensitization.

IV. RESULTS

Under the described conditions the following was recorded:

Preliminary study

The sample induced at the dosages (of 0,5 ml/animal) of the 100 % concentration and the 75 % dilution slight to moderate erythema.

Main study

On all animals no signs of erythema and edema were observed during the entire testing-period.

According to the method modified by Ritz, H. L. and Buehler, E. V., the test substance "E-2414.01" (BCM BTS 930) is considered to cause no delayed contact hypersensitivity.

V. PROTOCOLS

Table 1

Individual values — Preliminary study

Animal-No.	sex	concentration	24 h	48 h
1	♂	100 % (= undiluted)	2	2
2	♀	100 % (= undiluted)	1	2
3	♂	75 % in aqua dest.	1	1
4	♀	75 % in aqua dest.	1	1
5	♂	50 % in aqua dest.	0	0
6	♀	50 % in aqua dest.	0	0
7	♂	25 % in aqua dest.	0	0
8	♀	25 % in aqua dest.	0	0

Table 2

Individual values after challenge Main study

Testgroup (E-2414.01 50 %)

Animal-No.	sex	6 h	24 h	48 h
1	♂	0	0	0
2	♂	0	0	0
3	♂	0	0	0
4	♂	0	0	0
5	♂	0	0	0
6	♂	0	0	0
7	♂	0	0	0
8	♂	0	0	0
9	♂	0	0	0
10	♂	0	0	0
11	♀	0	0	0
12	♀	0	0	0
13	♀	0	0	0
14	♀	0	0	0
15	♀	0	0	0
16	♀	0	0	0
17	♀	0	0	0
18	♀	0	0	0
19	♀	0	0	0
20	♀	0	0	0

Table 3

Individual values after challenge Main study

Controlgroup (E-2414.01 50 %)

Animal-No.	sex	6 h	24 h	48 h
21	♂	0	0	0
22	♂	0	0	0
23	♂	0	0	0
24	♂	0	0	0
25	♂	0	0	0
26	♀	0	0	0
27	♀	0	0	0
28	♀	0	0	0
29	♀	0	0	0
30	♀	0	0	0

VI. GENERAL INFORMATION

Sponsor:	Procter & Gamble European Technical Center Temselaan 100 1820 Grimbergen (Strombeek-Bever)
Study performed by:	IBR Forschungs GmbH Südkampen Nr. 31 3030 Walsrode 1
Study director:	Dr. Dr. W. Sterner Fachtierarzt für Pharmakologie u. Toxikologie Expert agréé pharmacologue, toxicologue Fachtierarzt für klin. Laboratoriumsdiagnostik Fachtierarzt für Versuchstierkunde
Project leader:	Dr. med. vet. O. Chibanguza Fachtierarzt für Pharmakologie u. Toxikologie
Assistants:	Frau M. Fürst, Frl. N. Hindemann, Herr H. Quaren
Quality assurance.:	P. Volkmann
Time of study:	17.10. - 22.11.84
Archives and documents:	All raw data and a copy of the final report will be stored in the archives of IBR. The test substance was returned to the sponsor.

DECLARATION

The conditions for the performance of all studies, e.g. animal care, rooms, technical equipment and personnel are regularly controlled by the Quality Assurance Unit.

This report provides a correct and faithful record of the results obtained.

P. Volkmann .....

QAU

Södkampen, 30.11.84